

What is claimed is:

1. An isolated thioaptamer that mediates gene silencing.
2. The thioaptamer of claim 1, further comprising a terminal 3' hydroxyl group.
3. The thioaptamer of claim 1, wherein the thioaptamer comprises ribonucleotides.
- 5 4. The thioaptamer of claim 1, wherein the thioaptamer comprises deoxyribonucleotides.
5. The thioaptamer of claim 1, wherein the thioaptamer comprises one or more of the following: rATP(α S), rUTP(α S), rGTP(α S), rCTP(α S), rATP(α S₂), rUTP(α S₂), rGTP(α S₂) or rCTP(α S₂).
6. The thioaptamer of claim 1, wherein the thioaptamer comprises from about 21 to about
10 25 nucleotides.
7. The thioaptamer of claim 1, wherein the thioaptamer comprises a double stranded thioaptamer with a perfect complementarity match to a target gene and gene silencing occurs by mRNA cleavage.
8. The thioaptamer of claim 1, wherein the thioaptamer comprises an imperfect
15 complementarity match to a target gene and gene silencing occurs by repressed translation of mRNA to protein.
9. The thioaptamer of claim 1, wherein the thioaptamer comprises a single-stranded thioaptamer with perfect complementarity match to a target gene and gene silencing occurs by mRNA cleavage.
- 20 10. The thioaptamer of claim 1, wherein the thioaptamer comprises a portion of a RNA-induced silencing complex (RISC) complex.
11. The thioaptamer of claim 1, wherein the thioaptamer is produced by a DICER complex.
12. The thioaptamer of claim 1, wherein the thioaptamer comprises a short interfering RNA (siRNA); a micro, interfering RNA (miRNA); a small, temporal RNA (stRNA); or a short,
25 hairpin RNA (shRNA).

13. The thioaptamer of claim 1, wherein the thioaptamer is further defined as a thioaptamer precursor that comprises a long dsRNA, an about 70 nucleotide stem-loop RNA (shRNA) or an about 70 nucleotide stem-loop RNA (shRNA).
14. The thioaptamer of claim 1, wherein the thioaptamer comprises a double stranded
5 thioaptamer of about 21 to about 25 nucleotides long.
15. The thioaptamer of claim 1, wherein the thioaptamer comprises a single-stranded thioaptamer that is about 15 to about 22 nucleotides long.
16. The thioaptamer of claim 1, wherein gene silencing is defined further as degradation of an mRNA transcript that is cleaved in the presence of the thioaptamer before it can express a
10 protein.
17. The thioaptamer of claim 1, wherein gene silencing is defined further as regulation of translation when the thioaptamer binds an mRNA transcript at or about its 3'UTR.
18. A method of producing a mature thioaptamer of from about 21 to about 23 nucleotides in length comprising the steps of:
- 15 combining a double-stranded precursor thioaptamer with a soluble extract that mediates gene silencing, thereby producing a precursor-extract mixture; and
- maintaining the precursor-extract mixture under conditions in which the double-stranded thioaptamer is processed to the mature thioaptamer of from about 21 to about 23 nucleotides in length.
- 20 19. The method of claim 18, further comprising isolating the thioaptamer of from about 21 to about 23 nucleotides from the precursor-extract mixture.
20. The method of claim 18, further comprising the step of determining the sequence of the mature thioaptamer and the location of one or more thio-modifications to the mature thioaptamer.
21. The method of claim 18, further comprising the steps of:
- 25 determining the sequence of the mature thioaptamer and the location of one or more thio-modifications to the mature thioaptamer; and

chemically synthesizing the mature thioaptamer.

22. A mature thioaptamer of about 21 to about 23 nucleotides produced by the method of claim 18.

23. A method of mediating gene silencing of a target gene in a cell or organism comprising
5 the steps of:

introducing a thioaptamer of from about 21 to about 23 nucleotides in length into the cell or organism; and

maintaining the cell or organism under conditions in which gene silencing occurs, thereby mediating expression of the target gene in the cell or organism.

10 24. The method of claim 23, wherein thioaptamer is optimized for RNase H degradation of the message.

25. The method of claim 23, wherein the target gene encodes a viral gene.

26. The method of claim 23, wherein the target gene encodes a cellular gene.

27. The method of claim 23, wherein gene silencing is defined further as degradation of an
15 mRNA transcript of the target gene that is cleaved in the presence of the thioaptamer before it can express a protein;

28. The method of claim 23, wherein gene silencing is defined further as regulation of translation of the target gene when the thioaptamer binds an mRNA transcript of the target gene at or about its 3'UTR.

20 29. The method of claim 23, wherein the thioaptamer comprises a double stranded thioaptamer with a perfect complementarity match to the target gene and gene silencing occurs by mRNA cleavage.

30. The method of claim 23, wherein the thioaptamer comprises an imperfect
25 complementarity match to the target gene and gene silencing occurs by repressed translation of mRNA to protein.

31. The method of claim 23, wherein the thioaptamer comprises a single-stranded thioaptamer with perfect complementarity match to the target gene and gene silencing occurs by mRNA cleavage.
32. A knockdown cell or organism generated by the method of claim 23.
- 5 33. The knockdown cell or organism of claim 32, wherein the cell or organism mimics a disease.
34. The knockdown cell or organism of claim 32, wherein the cell comprises a stem cell.
35. A method of examining the function of a gene in a cell or organism comprising the steps of:
- 10 introducing a thioaptamer of from about 21 to about 23 nucleotides that targets an mRNA of the gene for gene silencing into the cell or organism, thereby producing a test cell or test organism; maintaining the test cell or test organism under conditions under which gene silencing of mRNA of the gene occurs, thereby producing a test cell or test organism in which mRNA of the gene is silenced; and
- 15 observing the phenotype of the test cell or test organism against an appropriate control cell or control organism to provide information about the function of the gene.
36. A method of assessing whether a gene product is a suitable target for drug discovery comprising the steps of:
- 20 introducing an RNA thioaptamer that mediates gene silencing of from about 21 to about 25 nucleotides into a cell or organism under conditions in which gene silencing of an mRNA for the target gene results in decreased expression of the gene; and
- determining the effect of the decreased expression of the gene on the cell or organism, wherein if decreased expression has an effect, then the gene product is a target for drug discovery.
37. A pharmaceutical composition comprising a thioaptamer of from about 21 to about 25
25 nucleotides that mediates thioaptamer gene silencing and an appropriate carrier.

38. A method of identifying target sites within an mRNA that are efficiently targeted for gene silencing, comprising the step of:

combining an RNA thioaptamer corresponding to a sequence of a labeled mRNA to be degraded under conditions in which labeled mRNA is degraded.

5 39. The method of claim 38, further comprising the step of identifying one or more sites in the mRNA that are efficiently cleaved.

40. The method of claim 38, wherein the RNA thioaptamer is defined further as a thioaptamer library.

10 41. The method of claim 38, wherein the RNA thioaptamer is defined further as a pool of thioaptamers from a thioaptamer library.

42. A method of identifying target sites within an mRNA that are efficiently targeted for gene silencing, comprising the step of:

combining an RNA thioaptamer corresponding to a sequence of a labeled mRNA under conditions in which labeled mRNA is not degraded and the protein level is reduced.

15 43. The method of claim 42, wherein the RNA thioaptamer is defined further as a thioaptamer library.

44. The method of claim 42, wherein the RNA thioaptamer is defined further as a pool of thioaptamers from a thioaptamer library.

45. A combinatorial thioaptamer library comprising:

20 two or more unique thioaptamers that comprise a combination of backbone modifications and sequence that mediates gene silencing of an mRNA to which it corresponds.

46. The library of claim 45, wherein the thioaptamers are attached covalently to one or more beads.

25 47. The library of claim 46, wherein the beads are polystyrene/polydivinyl benzene copolymer.

48. The library of claim 45, wherein the thioaptamers comprise one or more phosphorothioate linkages.
49. The library of claim 45, wherein the thioaptamers comprise one or more phosphorodithioate linkages.
- 5 50. The library of claim 45, wherein the thioaptamers comprise one or more methylphosphonate linkages.
51. The library of claim 45, wherein the thioaptamers comprises one or more of the following: rATP(α S), rUTP(α S), rGTP(α S), rCTP(α S), rATP(α S₂), rUTP(α S₂), rGTP(α S₂) and rCTP(α S₂).
- 10 52. The library of claim 45, wherein the thioaptamer comprises a viral protein sequence.
53. The library of claim 45, wherein the thioaptamer comprises a genomic sequence.
54. The library of claim 45, wherein the thioaptamer comprises an expressed sequence.
55. The library of claim 45, wherein each of the thioaptamers further comprise a colorimetric agent.
- 15 56. The library of claim 45, further comprising the complementary strand to the thioaptamer.
57. The library of claim 45, wherein the thioaptamers is created by a split and pool combinatorial synthesis chemistry.
58. The library of claim 45, wherein the thioaptamer library comprises double stranded thioaptamers with a perfect complementarity match to a target gene and gene silencing occurs by
20 mRNA cleavage.
59. The library of claim 45, wherein the thioaptamer library comprises thioaptamers with imperfect complementarity matches to a target gene and gene silencing occurs by repressed translation of mRNA to protein.
60. The library of claim 45, wherein the thioaptamer comprises library single-stranded
25 thioaptamers with a perfect complementarity match to a target gene and gene silencing occurs by mRNA cleavage.

61. A one-bead, one-thioaptamer combinatorial library comprising:

two or more beads, wherein attached to each bead is a unique thioaptamer comprising a single unique sequence, wherein each unique thioaptamer comprises a unique mix of modified and unmodified nucleotides and wherein the thioaptamer mediates gene silencing of an mRNA to which it corresponds.

62. A one-bead, one-thioaptamer combinatorial library comprising:

two or more beads, wherein attached to each bead is a unique thioaptamer comprising an imperfect complementarity match to a target gene to form a thioaptamer-bead, wherein each unique thioaptamer-bead comprises a unique mix of modified and unmodified nucleotides and wherein the thioaptamer mediates gene silencing of an mRNA to which it has imperfect complementarity.

62. A combinatorial library comprising:

a bead library of thioaptamer libraries, wherein each bead comprises a thioaptamer library of imperfect complementarity to a target sequence for gene silencing.

63. A method for reducing the expression of a gene in a cell, comprising the steps of:

selecting a thioaptamer that mediates gene silencing of the gene to which it corresponds; and introducing the thioaptamer into the cell, wherein the thioaptamer mediates RNA interference of a targeted sequence.

64. The method of claim 63, wherein the thioaptamer comprises from about 21 to about 25 nucleotides.

65. The method of claim 63, wherein the thioaptamer comprises a double stranded thioaptamer with a perfect complementarity match to a target gene and gene silencing occurs by mRNA cleavage.

66. The method of claim 63, wherein the thioaptamer comprises an imperfect complementarity match to a target gene and gene silencing occurs by repressed translation of mRNA to protein.

67. The method of claim 63, wherein the thioaptamer comprises a single-stranded thioaptamer with perfect complementarity match to a target gene and gene silencing occurs by mRNA cleavage.
68. The method of claim 63, wherein the thioaptamer comprises a portion of a RNA-induced silencing complex (RISC) complex.
69. The method of claim 63, wherein the thioaptamer is produced by a DICER complex.
70. The method of claim 63, wherein the thioaptamer comprises a short interfering RNA (siRNA); a micro, interfering RNA (miRNA); a small, temporal RNA (stRNA); or a short, hairpin RNA (shRNA).
71. The method of claim 63, wherein the thioaptamer is further defined as a thioaptamer precursor that comprises a long dsRNA, an about 70 nucleotide stem-loop RNA (shRNA) or an about 70 nucleotide stem-loop RNA (shRNA).
72. The method of claim 63, wherein the targeted sequence is selected from the group consisting of: markers, splice acceptors, splice donors, IRES, recombinase sites, promoters, ori sequences, cloning sites, and intervening sequence.
73. The method of claim 63, wherein the targeted sequence comprises a viral sequence.
74. The method of claim 63, wherein the targeted sequence comprises an autologous sequence.
75. The method of claim 63, wherein the targeted sequence comprises a heterologous sequence.
76. The method of claim 63, wherein the cell is a mammalian cell.
77. The method of claim 63, wherein the cell is a human cell.
78. The method of claim 63, wherein the cell is a stem cell.
79. The method of claim 63, wherein the thioaptamer is an antisense molecule.
80. The method of claim 63, wherein the thioaptamer is a ribozyme.

81. The method of claim 63, wherein the thioaptamer is a double-stranded RNA (dsRNA).
82. The method of claim 63, wherein the gene is associated with a disease or disorder.
83. A method for attenuating expression of a target gene in cultured cells, comprising the step of:
- 5 introducing an RNA thioaptamer into the cells in an amount sufficient to attenuate expression of the target gene, wherein the RNA thioaptamer comprises a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence of the target gene and mediates attenuation of protein expression for a gene to which it corresponds.
84. The method of claim 83, wherein the cell is in cell culture.
- 10 85. The method of claim 83, wherein the cell is infected with a virus.
86. The method of claim 83, wherein the cell is a mammalian cell.
87. The method of claim 83, wherein the cell is a human cell.
88. The method of claim 83, wherein the cell is a stem cell.
89. The method of claim 83, wherein the thioaptamer is an antisense molecule.
- 15 90. The method of claim 83, wherein the thioaptamer is a ribozyme.
91. The method of claim 83, wherein the thioaptamer is a double-stranded RNA (dsRNA).
92. The method of claim 83, wherein the gene is associated with a disease or disorder.
93. A method for attenuating expression of a target gene in a mammalian cell, comprising the steps of:
- 20 introducing into the cell a thioaptamer in an amount sufficient to attenuate expression of the target gene, wherein the thioaptamer mediates gene silencing of a nucleic acid to which it hybridizes under stringent conditions; and
- activating a gene silencing activity in the cell.
94. The method of claim 93, wherein the cell is in cell culture.

95. The method of claim 93, wherein the cell is in an animal.